Short-course postexposure antibiotic prophylaxis combined with vaccination protects against experimental inhalational anthrax

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14. ABSTRACT

Prevention of inhalational anthrax after Bacillus anthracis spore exposure requires a prolonged course of antibiotic prophylaxis. In response to the 2001 anthrax attack in the United States, approximately 10,000 people were offered 60 days of antibiotic prophylaxis to prevent inhalational anthrax, but adherence to this regimen was poor. We sought to determine whether a short course of antibiotic prophylaxis after exposure could protect non-human primates from a high-dose spore challenge if vaccination was combined with antibiotics. Two groups of 10 rhesus macaques were exposed to approximately 1,600 LD50 of spores by aerosol. Both groups were given ciprofloxacin by orogastric tube twice daily for 14 days, beginning 1-2 h after exposure. One group also received three doses of the licensed human anthrax vaccine (anthrax vaccine adsorbed) after exposure. In the ciprofloxacin-only group, four of nine monkeys (44%) survived the challenge. In contrast, all 10 monkeys that received 14 days of antibiotic plus anthrax vaccine adsorbed survived (P = 0.011). Thus postexposure vaccination enhanced the protection afforded by 14 days of antibiotic prophylaxis alone and completely protected animals against inhalational anthrax. These data provide evidence that postexposure vaccination can shorten the duration of antibiotic prophylaxis required to protect against inhalational anthrax and may impact public health management of a bioterrorism event.

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Bacillus anthracis, anthrax, antibiotics, ciprofloxacin, dose estimation, laboratory animals, nonhuman primates

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Short-course postexposure antibiotic prophylaxis combined with vaccination protects against experimental inhalational anthrax

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Prevention of inhalational anthrax after *Bacillus anthracis* spore exposure requires a prolonged course of antibiotic prophylaxis. In response to the 2001 anthrax attack in the United States, ≈10,000 people were offered 60 days of antibiotic prophylaxis to prevent inhalational anthrax, but adherence to this regimen was poor. We sought to determine whether a short course of antibiotic prophylaxis after exposure could protect non-human primates from a high-dose spore challenge if vaccination was combined with antibiotics. Two groups of 10 rhesus macaques were exposed to ≈1,600 LD_{50} of spores by aerosol. Both groups were given ciprofloxacin by orogastric tube twice daily for 14 days, beginning 1–2 h after exposure. One group also received three doses of the licensed human anthrax vaccine (anthrax vaccine adsorbed) after exposure. In the ciprofloxacin-only group, four of nine monkeys (44%) survived the challenge. In contrast, all 10 monkeys that received 14 days of antibiotic plus anthrax vaccine adsorbed survived ($P = 0.011$). Thus postexposure vaccination enhanced the protection afforded by 14 days of antibiotic prophylaxis alone and completely protected animals against inhalational anthrax. These data provide evidence that postexposure vaccination can shorten the duration of antibiotic prophylaxis required to protect against inhalational anthrax and may impact public health management of a bioterrorism event.

Bacillus anthracis | treatment | vaccine

Bacillus anthracis infection in humans occurs as cutaneous, gastrointestinal, or inhalational anthrax depending upon the route of exposure. Cutaneous anthrax is rarely fatal and can be effectively treated with antibiotics. Inhalational anthrax, the form likely to occur after a bioterrorist attack, on the other hand, is difficult to diagnose early, and despite antibiotic therapy, has a high fatality rate. Anthrax is rare in industrialized countries, and vaccination with anthrax vaccine adsorbed (AVA) is confined to those who could be potentially exposed to anthrax, such as veterinary workers, woolen mill employees, and laboratory workers (1). Military personnel in the United States are also vaccinated due to the potential threat of *B. anthracis* being used as a bioweapon.

Past experiments have shown that the rhesus macaque is the animal model that most closely mimics inhalational anthrax in humans (2). In both humans and macaques, inhalational anthrax begins with the deposition of 1- to 5-μm spores in the alveolar spaces, where spores are thought to be ingested by alveolar phagocytic cells. Some spores survive inside the phagocyte and are transported to the draining pulmonary and mediastinal lymph nodes where germination occurs. Although most spores probably germinate within a few days after inhalation, germination is not synchronous (3). Some spores remain dormant and do not germinate for prolonged periods (4, 5). It is the delayed germination of retained spores into vegetative bacilli that necessitates the prolonged use of prophylactic antibiotics after an inhalational exposure to *B. anthracis*. As first described by Barnes (6), antibiotics are active only after spores have germinated, and retained spores persisting after antibiotics are discontinued may subsequently germinate and thus cause disease. Animal experiments have confirmed the prolonged persistence of spores and incubation period after aerosol exposure (4, 5, 7). In one study, rhesus macaques were protected during a 30-day course of antibiotic prophylaxis after aerosol exposure. However, some animals developed fatal infection after the antibiotic therapy was discontinued (7).

The anthrax terrorist attacks in 2001 caused 11 cases of inhalational anthrax and placed many more persons at risk. The public health response to these events included an unprecedented prevention program in which ≈10,000 people were offered 60 days of postexposure antibiotic prophylaxis to prevent inhalational anthrax (8). Adverse events associated with antibiotic prophylaxis such as diarrhea, nausea, vomiting, and dizziness were commonly reported (9). More importantly, the overall adherence rate during 60 days of antibiotics was poor (44%; ref. 9). Despite these low adherence rates, there were no additional anthrax cases, suggesting that the doses of inhaled spores were probably very low. In contrast, computer modeling suggests that protection against higher doses of *B. anthracis* spores will require >4 months of antibiotic prophylaxis (10).

Combining prompt antibiotic prophylaxis and AVA vaccination, in theory, offers the best means of protecting individuals against inhalational anthrax, although the optimal duration of prophylaxis remains unclear. Minimizing the duration of postexposure antibiotic prophylaxis could be crucial to a successful defense against a large-scale bioterrorism attack. Using the rhesus macaque model of inhalational anthrax, we performed an experiment to test the hypothesis that adding AVA to a short course of antibiotic prophylaxis enhances survival and thus shortens the duration of postexposure antibiotic prophylaxis required for protection.

Results

As expected, all four untreated animals died 4–5 days (mean ± SD = 4.25 ± 1.29) after aerosol challenge with *B. anthracis* spores, similar to results reported in previous studies (4, 7). Three control animals were found dead, and the fourth was killed on day 5 after being found unresponsive. An antemortem

Conflict of interest statement: R.S. has a potential conflict of interest related to the fact that he is an employee of Bayer Pharmaceutical Corporation, which produces ciprofloxacin, a drug used in the study.

Abbreviation: AVA, anthrax vaccine adsorbed.

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Table 1. Survival after postexposure prophylaxis of inhalational anthrax

Treatment	Survivors/total
Ciprofloxacin	4/9*†
Ciprofloxacin plus vaccine	10/10†
Control untreated	0/4

*One animal died on study day 23 from aspiration pneumonia, not anthrax. This animal was excluded from the primary statistical analysis.
†$P = 0.011$, Fisher's exact test.

blood culture of this killed animal demonstrated 5.7×10^5 colony-forming units of *B. anthracis* per milliliter of blood. At necropsy, all animals in the control group had positive cultures of blood, spleen, lung, brain, and lymph nodes for *B. anthracis* and histological evidence of necrohemorrhagic mediastinal lymphadenitis. Three of four animals also had evidence of meningitis.

In contrast to the untreated control animals, all animals in both the ciprofloxacin-only and the ciprofloxacin-plus-vaccine groups remained well and survived during the 14 days of antibiotic prophylaxis. However, once the antibiotics were discontinued, only four of nine animals (44%) in the ciprofloxacin-only group survived compared with 10 of 10 animals (100%) that received ciprofloxacin plus AVA ($P = 0.011$, Table 1). The deaths of animals in the ciprofloxacin-only group occurred 5–10 days (mean ± SD = 7.2 ± 2.2) after finishing the 14-day course of antibiotic prophylaxis. Three of five animals in the ciprofloxacin-only group that died were found dead, whereas the remaining two animals were killed. Antemortem blood cultures of these two killed animals demonstrated 1.6×10^6 and 1.0×10^8 colony-forming units of *B. anthracis* per milliliter of blood. All five animals in the ciprofloxacin-only group that died of anthrax had positive cultures of blood, spleen, lung, brain, and lymph nodes for *B. anthracis* and necrohemorrhagic mediastinal lymphadenitis and meningitis at necropsy.

One animal in the ciprofloxacin-only group died on day 23 of causes unrelated to anthrax. Necropsy of this animal found aspiration pneumonia as the cause of death. Culture of this animal's lung demonstrated a small number of *B. anthracis* organisms. However, cultures of the animal's blood, spleen, brain, and mediastinal lymph nodes were all negative. This animal was eliminated from the statistical analysis, because it was impossible to determine whether it would have died from anthrax. If this animal had eventually survived and was included in the analysis, the overall survival would be 5 of 10 with $P = 0.033$ compared with the ciprofloxacin-plus-vaccine group. If the animal had eventually died of anthrax and was included in the analysis, the overall survival would be 4 of 10 with $P = 0.011$ compared with the ciprofloxacin-plus-vaccine group. Thus, the difference in survival between the ciprofloxacin-only vs. ciprofloxacin-plus-vaccine groups would be statistically significant in all cases.

The overall course and survival of the ciprofloxacin-only and ciprofloxacin-plus-vaccine groups are shown in Fig. 1. A log-rank test comparison of the Kaplan–Meier survival analysis showed that survival was significantly greater in the ciprofloxacin-plus-vaccine group ($P = 0.0069$). All surviving animals have continued to remain free of any signs of illness as long as 150 days after challenge.

Discussion

The 2001 anthrax terrorist attacks highlighted a unique problem associated with the medical management of aerosol exposure to *B. anthracis* spores. Because of the delayed germination of spores into vegetative bacilli, protection requires a prolonged course of antibiotic prophylaxis. In this study, we demonstrate that the addition of vaccination to a short course of antibiotics after lethal aerosol spore exposure significantly enhanced the protection afforded by antibiotics alone.

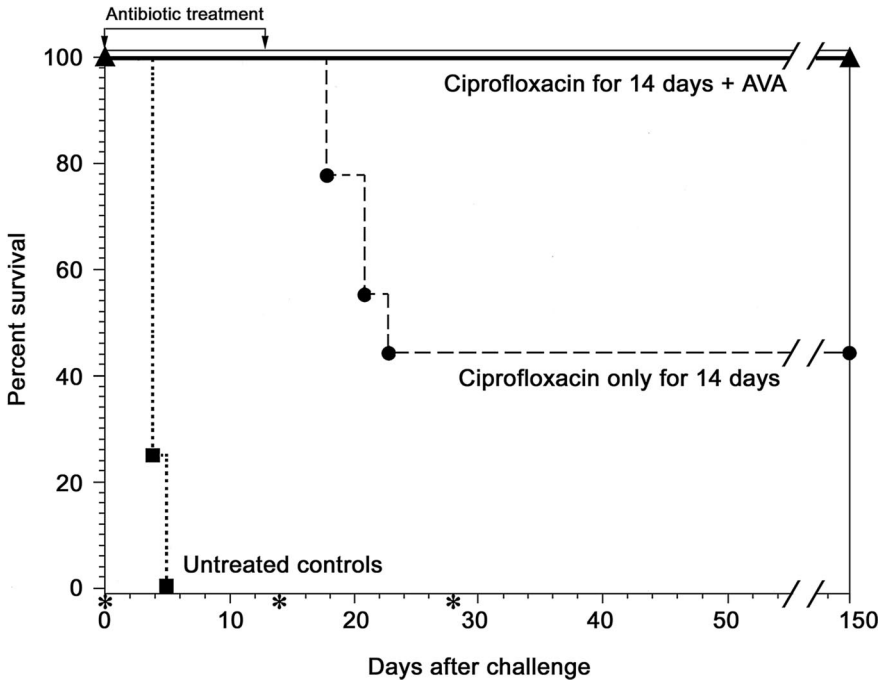

Fig. 1. Effect of vaccination combined with postexposure antibiotic prophylaxis on survival from inhalational anthrax. Animals received 14 days of ciprofloxacin alone after exposure (●, $n = 9$), 14 days of ciprofloxacin plus AVA after exposure (▲, $n = 10$), or no treatment (■, $n = 4$). Antibiotic postexposure prophylaxis was given from day 0 to 13 (↓). AVA was given on days 0, 14, and 28 (*). Analysis of the Kaplan–Meier survival curves showed that the probability of survival was significantly greater in the ciprofloxacin-plus-AVA than in the ciprofloxacin-only group ($P = 0.0069$).

An additional approach to postexposure prophylaxis involves combining antitoxin antibodies with antibiotics to target both the toxin and the organism. In a study using an i.p. spore challenge in mice, the combination of ciprofloxacin and polyclonal antibodies to the protective antigen component of the toxin gave increased protection when compared with treatment with ciprofloxacin or antibodies alone (11). However, the addition of antitoxin antibodies would not be optimal for the prevention of anthrax resulting from the residual spores that may germinate after discontinuation of the antibiotic, and repeated doses of antibodies may be required. The active immunity induced by postexposure vaccination would be preferred to the passive immunity provided by antibodies.

The present study was designed to mimic several key variables relevant to a public health emergency involving an inhalational anthrax bioterrorism event. In such a scenario, oral antibiotics would be the only efficient way to deliver antimicrobial therapy to large numbers of individuals quickly. Thus, each animal was given 125 mg of ciprofloxacin every 12 h, an oral dose previously shown to provide therapeutic antibiotic levels (12). This was confirmed in the present study in which peak-and-trough serum samples from all animals in both groups had ciprofloxacin concentrations above the minimum inhibitory concentration for *B. anthracis* (0.06 μg/ml; data not shown). Analysis of serum ciprofloxacin levels demonstrated no significant differences between the ciprofloxacin-only and the ciprofloxacin-plus-vaccine groups in their peak ($P = 0.737$) or trough ($P = 0.623$) levels. In the ciprofloxacin-only group, there were no significant differences between animals that survived and those that succumbed to anthrax in their peak ($P = 0.112$) or trough ($P = 0.914$) serum ciprofloxacin levels (data not shown), so that the difference in survival was not due to differences in antibiotic pharmacokinetics.

We also exposed the non-human primates to a very large aerosol dose of >1,000 LD_{50} to simulate potentially heavy exposures that may be associated with a bioterrorism event. Even with this large challenge dose, combining postexposure antibiotics and AVA completely protected the animals from dying from inhalational anthrax.

It was shown previously that in rhesus macaques challenged with a relatively low (8 LD_{50}) aerosol dose of *B. anthracis* spores, 30 days of postexposure antibiotic prophylaxis prevented infection and the development of a protective immune response, as indicated by the failure of those animals to develop antibodies to the protective antigen component of anthrax toxin (7). In this study, in contrast, all surviving animals in the antibiotic-only group developed measurable IgG antibody titers to protective antigen on study day 23 (data not shown), indicating they became infected. The development of infection suggests that some proliferation of organisms occurs after a very high spore dose challenge, even when treatment is begun early after exposure. Despite this evidence for active infection, none of the animals showed clinical signs of being infected. Our observations of survival and the development of an immune response after a short course of postexposure antibiotic prophylaxis may have implications for the treatment of established disease as well. Infected individuals presenting with symptomatic inhalational anthrax who are treated, recover, and seroconvert may not require 60 days of antibiotic therapy, as recommended by the current guidelines (13), although additional research is warranted.

To summarize, rhesus macaques exposed to a high dose of aerosolized *B. anthracis* spores and administered 14 days of postexposure ciprofloxacin experienced a high mortality rate after antibiotics were discontinued. Adding AVA to postexposure antibiotic prophylaxis resulted in a statistically significant increase in survival ($P = 0.011$). Shortening the duration of antibiotic postexposure prophylaxis in a bioterrorism event involving *B. anthracis* by adding postexposure vaccination could greatly alleviate problems of noncompliance and side effects associated with prolonged antibiotic therapy. The value of adding vaccination to postexposure antibiotic prophylaxis should be considered in planning the public health response to bioterrorism events involving inhalational anthrax.

Materials and Methods

Bacterial Strain Preparation and Aerosol Exposure. *B. anthracis* spores (Ames strain, U.S. Army Medical Research Institute of Infectious Diseases collection) were produced and purified according to previously published methods (14). The rhesus monkeys were anesthetized and exposed in a head-only chamber to a *B. anthracis* spore aerosol, as described (14). The animals were exposed to an inhaled mean dose of 1,646 (range 556–3,573) LD_{50}. One aerosol LD_{50} in the rhesus macaque corresponds to 5.5×10^4 spores (14).

Experimental Groups. Twenty adult rhesus macaques (*Macaca mulatta*) with a mean weight of 7.45 kg (range 3.7–14.1 kg) were randomly distributed by sex into two groups of 10 animals. Four additional animals were used as untreated controls. Postexposure antibiotic therapy with ciprofloxacin began in both treatment groups starting 1–2 h after aerosol exposure and continued twice daily for 14 days. Both groups of 10 animals received an initial loading dose of 250 mg of ciprofloxacin beginning within 2 h after aerosol exposure followed by 14 days of ciprofloxacin by orogastric tube (125 mg every 12 h), as described (12). Each animal in antibiotic-only and antibiotic-plus-vaccine groups received the same dose of ciprofloxacin. One group of 10 animals was also vaccinated with AVA (0.5 ml) s.c. on day 0 beginning 2 h after aerosol exposure and on days 14 and 28. The second group of 10 animals received ciprofloxacin only.

Clinical, Microbiology, and Pathology Studies. All animals that died were necropsied. Animals that were moribund were first anesthetized before being killed. Samples of blood, spleen, lungs, brain, and lymph nodes from all expired animals were cultured for bacteria. Tissues collected for histopathology were immersion-fixed in 10% neutral buffered formalin. Formalin-fixed tissues were stained routinely with hematoxylin/eosin. Selected tissues were stained with Lillie-Twort (Gram) stain.

Serum anti-IgG antibodies to the protective antigen component of the *B. anthracis* toxin were measured on study days 14, 17, 23, and 30, with an ELISA (15). Serum ciprofloxacin levels were measured by LC/tandem MS using a Sciex API 3000 (Applied Biosystems). Serum samples for peak ciprofloxacin levels were obtained 1 h after the antibiotic dose on days 2, 7 or 8, and 13. Serum samples for trough ciprofloxacin levels were obtained just before the antibiotic dose on days 3, 6, and 14.

This study was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals, and with adherence to principles stated in the *Guide for the Care and Use of Laboratory Animals* (16). The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Statistical Analysis. The significance of the difference in survival between the ciprofloxacin-only and ciprofloxacin-plus-vaccine groups was determined by using a two-tailed Fisher's exact test. Mean survival times were estimated by Kaplan–Meier survival analysis and log-rank tests. Differences in ciprofloxacin levels between the treatment groups were assessed for statistical significance by a repeated-measure ANOVA. The influence of ciprofloxacin levels on outcome within the ciprofloxacin-only group was analyzed by logistic regression. Analyses were conducted by using SAS Ver. 9.1 (SAS Institute, Cary, NC).

Addendum. The four animals that survived the 14-day course of ciprofloxacin and developed an immune response to protective antigen were rechallenged 8–11 months after discontinuation of ciprofloxacin to determine whether they were resistant to reinfection. All animals survived aerosol challenge with at least 200 LD_{50} of spores (data not shown). These results suggest that surviving animals that develop an immune response subsequent to antibiotic prophylaxis or treatment of inhalational anthrax will be resistant to reinfection. This further suggests that, in managing postexposure prophylaxis in humans, the development of an immune response after prophylaxis with antibiotic alone or in conjunction with vaccination may be used to determine when antibiotics can be safely discontinued.

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